Balancing and Directional Selection at Exon-2 of the MHC *DQB1* Locus among Populations of Odontocete Cetaceans

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The diversity of exon-2 (peptide-binding region) of the *DQB1* locus (Class II, major histocompatibility complex, MHC) was investigated on an extended sample of populations of three focal cetacean species (two sibling delphinid species and another in the same family). We tested the hypothesis that dolphin populations with a worldwide distribution across different habitats and geographic regions will be under differential selective pressure by comparing DQB1 variation with variation at neutral markers and by investigating putative functional residues within the exon-2 sequence at the population level. Variation at the *DQB1* locus was not correlated to neutral differentiation (assessed by comparison with microsatellite DNA markers), and overall F_{ST} values were significantly lower for the MHC locus, consistent with expectations for balancing selection. Measures of heterozygosity and d_n/d_s ratios were also consistent with balancing selection. However, outliers in the F_{ST} comparisons and the analysis of putative functional residues suggested incidences of directional selection in local populations.

Introduction

The major histocompatibility complex (MHC) is a multigene family that codes for cell surface glycoproteins that bind peptides of processed foreign antigens and present them to T-lymphocytes. MHC class I and class II loci have been shown to be highly polymorphic in, for example, primate, rodent, pinniped, avian, and bovine species (Klein 1986; Udina et al. 1994; Trowsdale 1995; Ellegren et al. 1996; Nasir et al. 1997; Wagner et al. 1998; Chardon et al. 1999; Horin and Matiasovic 2002; Otting et al. 2002; Villegas-Castagnasso et al. 2003; Piertney and Oliver 2006). Furthermore, polymorphism in MHC genes is often greatest at the sites that specify the amino acids of the peptide binding region (PBR), the region that is responsible for peptide collection and presentation (Klein and Figueroa 1986). Two of the main reasons that MHC polymorphism has been attributed to frequency and/or overdominant selection are: 1) the high nonsynonymous (d_n) relative to synonymous (d_s) substitution rates in the PBR and 2) trans-species polymorphism (Nei and Rooney 2005).

Neutral theory predicts that genes under selection can behave as effectively neutral when populations are small (when $s < 1/2N_e$), and therefore, MHC alleles may experience periods of neutral evolution, during which genetic drift and mutation are more prominent in maintaining MHC polymorphism than selection. MHC population genetic studies on Scandinavian beavers (Ellegren et al. 1993), bighorn sheep (Boyce et al. 1997), Amerindians (Cerna et al. 1993; Valdes et al. 1999), Australian bush rats (Seddon and Baverstock 1999), Atlantic salmon (Bernatchez and Landry 2003), wolves (*Canus lupus*; Seddon and Ellegren 2004), mountain goats (*Oreamnos americanus*; Mainguy et al. 2007), and brown trout (*Salmo trutta*; Campos et al. 2006) among others show evidence for the influence of genetic drift, often together with evidence for balancing selection.

Landry and Bernatcez (2001) showed that in the Atlantic salmon, differences in the environment and geographical scales correlated with significant differences in MHC class II B allelic frequencies. However, overall F_{ST} values were not significantly different from values for microsatellite loci. They suggested that, although balancing selection was evident, the population structure inferred by MHC analysis was shaped more by genetic drift and migration than selection (Landry et al. 2001). Campos et al. (2006) reported similar results for brown trout. Seddon and Ellegren (2004) found significant differentiation at DQA, DQB1, and DRB1 loci ($F_{ST} = 0.251$–0.269) among wolves in similar habitats in Finland, Estonia, Latvia, and eastern Russia. However, they also showed that temporal changes in variability at these loci were for the most part consistent with neutral evolution. The authors suggest that this was due to fragmentation and consequent genetic drift (Seddon and Ellegren 2004). Hayashi et al. (2006) also found evidence for both balancing selection overall, and genetic drift in small, local populations for the DQB locus in the finless porpoise (*Neophocaena phocaenoides*).

Evidence for directional selection has come in part from the mapping of allelic substitutions onto the inferred structural model of the MHC molecule (Hughes et al. 1996; Ou et al. 1998; Cohen 2002). For example, a study on the effects of pollution on MHC variation in estuarine fish showed that the population that had adapted to severe chemical pollution had specific amino acid substitutions in the α-helix region (Cohen 2002). Furthermore, the fish from the unpolluted area also exhibited a significantly different pattern in the β-pleated sheet of the PBR (Cohen 2002). Functional analysis has also been used in human studies (with the potential to extend this work to nonhuman species), where human pathologies have been correlated to specific amino acid replacement and motif changes in the PBR among different populations (e.g., Nepom and Erlich 1991; Winchester 1994; Hill 1998; Ou et al. 1998). Evidence for positive selection based on geographic or temporal differences in allele frequencies has been reported for transporter associated with antigen processing (TAP) genes in brown trout (Jensen et al. 2008) and for class II MHC genes among populations of the Asian cygomolgus macaque (*Macaca fascicularis;* Bonhomme et al. 2007), among other studies. TAP proteins deliver cytosolic

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peptides to the endoplasmic reticulum where they associate with the MHC molecule for presentation and have been shown to coevolve with MHC Class I molecules (McCluskey et al. 2004).

Pathogens that affect marine mammals have been associated with the marine environment for long evolutionary periods (Howard et al. 1983; Kennedy 1990; Limpscomb et al. 1994; Higgins 2000). For example, in cetaceans, the divergence between different morbillivirus species and a hypothetical common terrestrial ancestor occurred millions of years ago (Barrett et al. 1993, 1995; Osterhaus et al. 1995). Cetaceans are warm blooded and breathe air like all mammals; however, they rely on the aquatic environment for their life needs. This may result in an interaction with both terrestrial and marine pathogen risks. In the last 20 years, thousands of marine mammals have died due to epizootics caused by viral infections (Van Bressem et al. 1999). In many cases, these are likely indigenous pathogens, though not all. The Canine Distemper Virus infection from dogs, which took place in 1986 at lake Baikal in Russia, had devastating effects on the freshwater Baikal seal population (Mamaev et al. 1996; Forsyth et al. 1998).

Killer whale (*Orcinus orca*) populations can be found across all major oceans in both polar and temperate waters and in particular in coastal areas of high productivity (Dahleim and Heyning 1999; Ford 2002; Hoelzel, Natoli, et al. 2002). The social associations formed by this species are very stable, and there are regional populations that are known to have persisted for decades (Ford et al. 1998; Ford 2002; Hoelzel, Natoli, et al. 2002). Sympatric populations of foraging specialists (different ecotypes pursuing fish vs. marine mammal prey) found in the eastern North Pacific differ in ecology, behavior, and distribution patterns and are genetically differentiated, as are populations of the same ecotype in parapatry and allopatry (Hoelzel and Dover 1991; Hoelzel, Dahleim, and Stern 1998; Ford and Ellis 1999; Hoelzel, Goldsworthy, and Fleischer 2002). Populations of the fish-eating ecotype have been referred to as "residents" and the marine mammal–eating ecotype as "transients," and this terminology will be used here. Genetic analyses have identified at least seven populations in the North Pacific (Hoelzel et al. 2007).

The bottlenose dolphin (*Tursiops truncatus*) is also found in all major oceans, from cold temperate to tropical seas, in coastal and offshore waters. *Tursiops truncatus* exhibits habitat differentiation among populations across its range, as sympatric or parapatric populations will use the coastal (nearshore) or the pelagic (offshore) environment (Hoelzel, Potter, and Best 1998; Hoelzel, Goldsworthy, and Fleischer 2002; Natoli et al. 2004). Studies on mtDNA and nuclear DNA (microsatellites) have shown that coastal and pelagic *T. truncatus* populations in the western North Atlantic are genetically differentiated (Hoelzel, Dahleim, et al. 1998). In addition, in South Indian and South Pacific coastal habitats, a smaller morphotype has been described as the "aduncus" form. An mtDNA study by Wang et al. (1999) demonstrated that the coastal aduncus form in China shows a reciprocally monophyletic relationship to the offshore populations of *T. truncatus*, supporting the classification of the aduncus form as a separate species, *Tursiops aduncus*. Further to this, Natoli et al. (2004) showed that the South African aduncus morphotype formed a monophyletic lineage separate from both *T. truncatus* and the Chinese aduncus type. Genetic differentiation between coastal and offshore populations (Hoelzel et al. 1998) and among putative populations in the eastern North Atlantic, Mediterranean, and Black Sea suggested differentiation determined by oceanic habitat boundaries (Natoli et al. 2005).

Here, we test the hypothesis that for these species (each of which show neutral genetic differentiation apparently driven by differences in habitat or foraging strategy) regional populations will show differential evidence of balancing or positive selection at the *DQB1* MHC locus. In support of this, we find evidence for MHC differentiation that is not consistent with isolation by distance models or differentiation patterns seen at presumably neutral microsatellite DNA loci. We also chose a specific set of residues within the PBR known to show adaptive differentiation in other species, and found patterns consistent with differential selection for some regional populations. The implication is that both balancing and local positive selection are important in determining the pattern of variation at this locus in these species.

Materials and Methods

Samples

Tissue samples were acquired from various sources and extracted to DNA by standard methods (see Natoli et al. 2004, 2005; Hoelzel et al. 2007 for details). Killer whale samples were from the "southern resident" population off Washington state (SR; $N = 33$; see Hoelzel et al. 2007), Alaskan residents off SE Alaska (AR; $N = 31$), Alaskan transients (AT; $N = 35$), Californian transients (CT; $N = 24$), the Bering Sea and Aleutians (BR; $N = 14$), and Iceland (IC; $N = 31$). *Tursiops truncatus* populations were from the Mediterranean Sea (MED; $N = 29$; see Natoli et al. 2004), Eastern North Atlantic (ENA; $N = 26$), Western North Atlantic pelagic (WNAP; $N = 25$), Western North Atlantic coastal (WNAC; $N = 27$), and the eastern North Pacific off southern California (ENP; $N = 15$). A *T. aduncus* population was sampled off South Africa (SAA; $N = 140$). Map locations are provided in figure 1.

Molecular Methods

The exon-2 PBR region was amplified using the primers (CTGGTAGTTGTGTCTGCACAC and CATGTGCTACTTCACCAACGG) developed by Tsuji et al. (1992). The reaction conditions were 10 mM Tris HCl (pH = 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 μM of each primer, 2 units of *Pfu Taq* polymerase (Promega, Southampton, UK), and 100–150 ng of template DNA in a 25-μl final volume. For screening by SSCP, 2 μl of denaturing loading buffer 95% (v/v) formamide, 0.1% (w/v) bromophenol blue, 0.1% (w/v) xylene cyanol, and 10 mM NaOH (Sigma–Aldrich, Gillingham, UK) were added to 2 µl of PCR product and were loaded on a nondenaturing acrylamide gel 6% (v/v) 49:1 acrylamide:bisacrylamide, 10% (v/v) glycerol, and 1× TBE for 6 h and 40 W migrations at 4 °C. The gel was incubated for

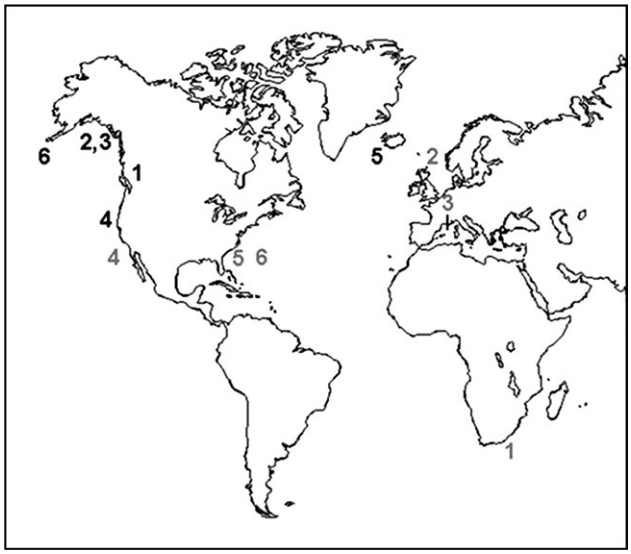

FIG. 1.—Sample locations for *Tursiops* sp. (in gray): 1: SAA, 2: ENA, 3: MED, 4: ENP, 5: WNAC, and 6: WNAP; for killer whales (in black): 1: SR, 2: AR, 3: AT, 4: CT, 5: IC, and 6: BR (see text for definition of abbreviations).

20 min with the fluorescent GelStar Nucleic Acid Gel Stain (BioWhittaker, Rockland, ME) according to manufacturer instructions. Allelic conformation was visualized by exposure to short-wave UV light and photographed. To confirm apparent genotypes and test for possible *Escherichia coli* recombinant artifacts (Longeri et al. 2002), up to 20 clones were rescreened by SSCP from different individuals and a subset sequenced in both directions (including the sequence of every allele for multiple individuals). Cloning was done using the Easy T-Vector Cloning kit (Promega) according to the manufacturer instructions. The polymerase chain reaction (PCR) fragments were purified using a PCR purification kit (Qiagen, Crawley, UK) and inserted into the *Eco*R1 site of the pGEM-T vector plasmid. Because the PCR fragments were generated using *Pfu* DNA polymerase (Promega) they were blunt ended, and therefore an A-Tail reaction was required. Sequencing was performed by the Big-Dye terminator reaction using the universal sequencing primers of the Easy T-Vector plasmid.

Structural Analysis

Human leukocyte antigen (HLA) *DR* (and *DQ*; Wecherpfennig and Strominger 1995) has identified subregions (referred to as pockets) in the binding groove, which influence binding, presentation, and recognition by T-cell receptors (Stern et al. 1994). Among these subregions, pocket P4 amino acid residues β70, β71, and β74 have been shown to play a significant role in determining T-cell recognition of the peptide–HLA complex (Olson et al. 1994; Stern et al. 1994; Ou et al. 1996). It has been shown through site-directed mutagenesis in *DQ* and *DR* alleles of HLA that selective peptide binding is greatly affected by the amino acid residues in pocket P4 and the consequent charge (Hammer et al. 1995; Wecherpfennig and Strominger 1995; Ou et al. 1996). Alleles have been grouped into seven different functional categories according to physicochemical polymorphisms of these residues, and their influences on T-cell receptor recognition (Ou et al. 1997, 1998). Ou et al. (1998) suggested that these seven categories can be combined into four groups based on the sum of the charges at the β70, β71, and β74 residues: a positively charged group (+), a negatively charged group (−), a dicharged group (+/−), and a neutral group (n). For example, the *DRB1**1117 allele exhibits the residues RRE in positions 70, 71, and 74, respectively. Arginine (R) is positively charged, and glutamic acid (E) is negatively charged and so this allele is classified in the dicharged functional group. When a charged amino acid is present among nonpolar and/or neutral amino acids, then the allele is classified according to the charged amino acid present. The charge of the amino acids was determined according to the following categorization (Ou et al. 1998): H, K, and R positive; D and E negative; and the rest neutral. Comparisons of charge profiles were done using contingency tables implemented in the program RxC (http://www.marksgeneticsoftware.net/; 20 batches, 2,500 replicates per batch). RxC employs the metropolis algorithm to obtain an unbiased estimate of the exact *P* value (see Raymont and Rousset 1995).

Population Genetic Analysis

The allele frequencies, allelic richness, and gene diversity index (H_s) of the *DQB1* locus for each of the populations were estimated using FSTAT version 2.9.3 (Goudet 2001). The expected allelic frequencies under neutrality were estimated by the Ewens–Watterson–Slatkin exact test using the program ARLEQUIN version 2.000 (Schneider et al. 2000). In addition, ARLEQUIN was used to perform the Mantel matrix correlation test, F_{ST} index, expected (H_e based on the Hardy–Weinberg equilibrium) and observed (H_o) genotype frequencies. Statistical significance was estimated by a Chi-square test ($P < 0.05$, after Bonferroni correction). The Nei–Gojobori method (implemented in MEGA) was used to estimate the d_n/d_s ratio within the PBR region of the *DQB1* sequence. F_{ST} values for the *DQB1* locus were compared with published data on 16 microsatellite DNA loci for the killer whale (Hoelzel et al. 2007) and nine microsatellite DNA loci for the bottlenose dolphin (Natoli et al. 2004, 2005). DISTLM v.5 (Anderson 2004) was used to perform a permutation test for the F_{ST} matrices corrected for ln transformed geographic distance (Anderson 2001). Bootstrapping (15,000 replications) was undertaken over all microsatellite DNA loci to calculate the 95% confidence intervals (CIs) around F_{ST} estimates (using FSTAT). MHC F_{ST} values outside the 95% CIs were considered significantly different from the estimates derived using microsatellites (after Landry and Bernatcez 2001).

F_{ST} values are correlated with heterozygosity levels, so that outliers from this relationship can suggest directional (F_{ST} higher than expected) or balancing selection (F_{ST} lower than expected; Beaumont and Nichols 1996). We tested this using FDIST (Beaumont and Nichols 1996) as implemented through LOSITAN (Antao et al. 2008). Simulations were run for 10,000 replications,

Table 1
Diversity and Selection Parameters for the Killer Whale Populations

	SR	AR	AT	CT	IC	BR
N	33	31	35	24	31	14
A	5	6	4	4	5	5
R	3.832	4.901	3.989	3.974	4.956	5.000
H_s	0.619	0.762	0.697	0.657	0.779	0.786
H_e	0.677	0.789	0.742	0.701	0.782	0.791
H_o	0.818	0.871	0.886	0.917	0.968	0.929
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fewo	0.388	0.248	0.311	0.352	0.231	0.237
Fewe	0.462	0.393	0.544	0.517	0.455	0.389
P value	0.631	0.315	0.026	0.079	0.006	0.012
F_{IS}	−0.322	−0.143	−0.271	−0.396	−0.242	−0.182
P value	0.002	0.048	<0.001	<0.001	0.002	0.030
d_n/d_s	2.45	2.93	3.07	3.07	1.84	2.80
P value	0.028	0.016	0.015	0.029	0.046	0.017

NOTE.—*A* = number of alleles, *R* = allelic richness, Fewe = expected *F*-value for the Ewens–Watterson neutrality test, and Fewo = the observed value. AR = Alaska residents, SR = Southern residents, CT = Californian transients, IC = Iceland, and BR = Berring Sea.

Table 2
Diversity and Selection Parameters for the Bottlenose Dolphin Populations

	SAA	ENA	MED	ENP	WNAC	WNAP
N	140	26	29	15	27	25
A	7	7	7	6	7	7
R	3.015	5.923	6.364	5.561	6.028	6.076
H_s	0.559	0.759	0.807	0.807	0.818	0.747
H_e	0.609	0.762	0.834	0.795	0.882	0.828
H_o	0.657	0.654	0.828	0.867	0.630	0.680
P value	0.012	0.012	<0.001	0.004	<0.001	<0.001
Fewo	0.442	0.257	0.207	0.231	0.200	0.270
Fewe	0.440	0.328	0.336	0.332	0.331	0.324
P value	0.887	0.125	0.013	0.139	0.059	0.109
F_{IS}	−0.175	0.139	−0.026	−0.074	0.231	0.089
P value	0.003	0.133	0.297	0.173	0.016	0.219
d_n/d_s	28.9	62.7	37.2	62.5	29.2	31.6
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

NOTE.—*A* = number of alleles, *R* = allelic richness, Fewe = expected *F*-value for the Ewens–Watterson neutrality test, and Fewo = the observed value. SAA = South African aduncus type, ENA = Eastern North Atlantic, MED = Mediterranean, ENP = Eastern North Pacific, WNAC = Western North Atlantic Coastal, and WNAP = Western North Atlantic Pelagic.

95% CI, and using the options for neutral and forced mean F_{ST}. Outlier microsatellite loci were omitted (one for each species), as suggested by the authors, though this made no difference to the position of the MHC locus relative to the confidence limits in either case (data not shown). An infinite allele model was assumed, but replications using the stepwise mutation model made no difference to the result (data not shown). Bottlenose dolphin comparisons using this test were for *T. truncatus* only.

Results

Tables 1 and 2 summarize the data on indicators of *DQB1* diversity and possible selection in each population for the killer whale (table 1) and bottlenose dolphins (table 2). All killer whale populations showed a significant excess of observed heterozygotes compared with Hardy–Weinberg expectations (with all F_{IS} values significantly negative), and the level of diversity was similar among populations. For the bottlenose dolphin, four out of six populations showed a significant deficit of heterozygotes, whereas 2 showed significant excess. Evidence for balancing selection based on the Ewens–Watterson neutrality test has low power, but three populations were significant at the $P < 0.05$ level for the killer whale and one for the bottlenose dolphin. All populations of both species showed a PBR d_n/d_s ratio that was significantly greater than 1, with the bottlenose dolphin populations showing the strongest effect (tables 1 and 2). The bottlenose dolphin sample is represented by two sibling species, *T. truncatus* (represented by five populations) and *T. aduncus* (represented by one population). The latter is well sampled ($N = 140$) and provides clear evidence for heterozygote excess, whereas the smaller *T. truncatus* samples ($N = 15$–29) are more variable, and some may be impacted by sampling effects. At the same time, allelic richness is twice as high in most *T. truncatus* populations compared with *T. aduncus*.

Comparing against published microsatellite DNA data for the killer whale (Hoelzel et al. 2007) and the bottlenose dolphin (Natoli et al. 2005; fig. 2) shows the lack of correlation between F_{ST} values derived from microsatellite loci and the MHC data generated in this study (comparing the same populations; Mantel tests were nonsignificant for all killer whale populations together, as well as for just the fish-eating ecotype on its own; *T. truncatus* populations were tested omitting the one *T. aduncus* population). In figure 2 (and in tables 3 and 4), all nonzero values were significantly different from zero. The multiple regression data run using DISTLM made similar comparisons but corrected for geographic distance, and also found no significant relationship (for the killer whale: pseudo-$F = -0.009$, permutation $P = 0.389$; for the bottlenose dolphin: pseudo-$F = 0.091$, permutation $P = 0.957$). A pattern of isolation by distance based only on microsatellite DNA data has been previously shown for both species (Hoelzel et al. 2007; Nichols et al. 2007). Comparisons between *T. truncatus* populations and *T. aduncus* had uniformly high F_{ST} values for the microsatellite loci (see table 4, fig. 2), whereas MHC values were more valuable, including a low value between ENP and SAA (table 4). MHC F_{ST} values among *T. truncatus* populations were relatively low and invariant over a broad range of microsatellite F_{ST} values (table 4, fig. 2). Relatively low MHC F_{ST} values were seen for some comparisons over a broad range of microsatellite F_{ST} values for the killer whale populations as well (table 3, fig. 2). The overall MHC F_{ST} value (KW: 0.097; *T. truncatus*: 0.022) was significantly lower than the microsatellite DNA value for both species (KW: 95% CI = 0.098–0.172; *T. truncatus*: 95% CI = 0.110–0.314; data from Hoelzel et al. 2007; Natoli et al. 2004). For the killer whale MHC values, two pairwise comparisons against the SR population were above the microsatellite DNA F_{ST} CI range (see table 3). The test using FDIST provided no further evidence in support of selection for the killer whale (data not shown), but the position of the DQB locus for *T. truncatus* was highly significantly below the lower confidence limit ($P = 0.0$; fig. 3).

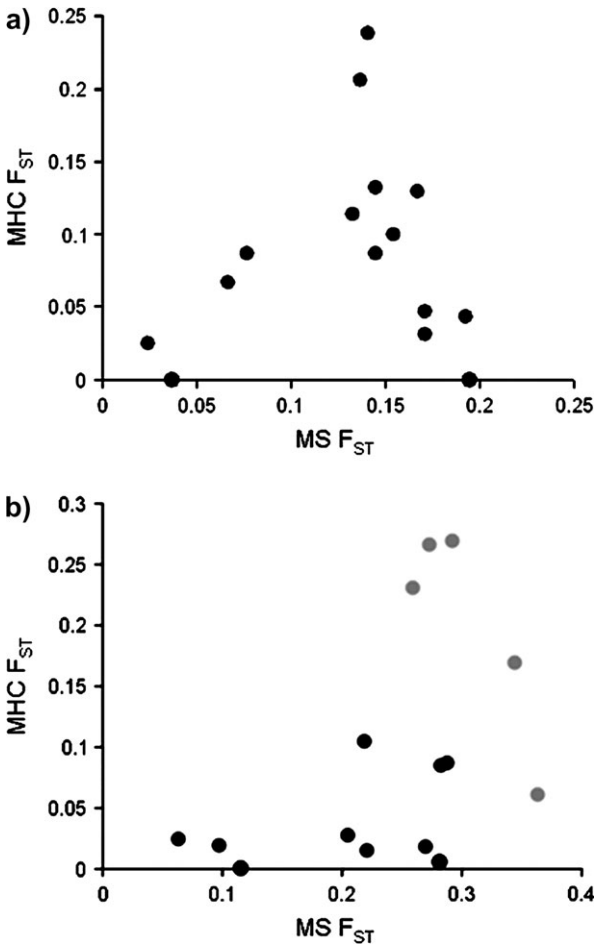

FIG. 2.—Correlation between F_{ST} values from MHC versus microsatellite DNA data for (*a*) killer whale and (*b*) bottlenose dolphins (comparisons between *Tursiops aduncus* and *Tursiops truncatus* shown in grey.

An assessment of possible functional differences among populations was conducted using the method of Ou et al. (1998). In Pocket P4 of the PBR of the HLA *DRB1* locus, 50% of the alleles are classified as dicharged (+/−), 39% as positive (+), 13% as negative (−), and 8% as neutral (n) (Ou et al. 1998). For our study species, charge-state proportions are illustrated by population in figure 4. Some highly differentiated populations (based on published mtDNA and microsatellite DNA data; Natoli et al. 2005; Hoelzel et al. 2007) show no significant difference for charge profiles (including the comparison between nearshore *T. truncatus* populations either side of the North Atlantic, and comparisons between killer whale populations between the North Pacific and North Atlantic), whereas some other profiles are distinct. The South African *T. aduncus* population had a different profile from all others (closest pattern was for ENP; SAA compared with ENP $P < 0.00001$). The ecotype of the small ENP sample is not known, and the ENA sample includes both coastal and likely offshore animals (from strandings). However, the WNAC and MED samples are known to be from coastal habitat, and there are data to suggest similar pathogen environments (see below). If these are combined and compared against the one offshore population sample (WNAP), those profiles are significantly different ($P = 0.048$). Other comparisons among *T. truncatus* populations were not significantly different. For the killer whale, the SR population shows a different proportion compared with AR ($P = 0.032$), and much stronger significant differentiation from all other populations ($P = 0.002$ to $P < 0.00001$). None of the other killer whale population profiles were significantly different from each other.

Table 4
F_{ST} Values for Microsatellite DNA Loci (above Diagonal; from Natoli et al. 2004) and the DQB Locus (below the Diagonal) for the Bottlenose Dolphin

	SAA	ENA	MED	ENP	WNAC	WNAP
SAA		0.273	0.293	0.364	0.354	0.260
ENA	0.266		0.098	0.288	0.282	0.116
MED	0.269	0.019		0.283	0.221	0.064
ENP	0.060	0.086	0.084		0.270	0.219
WNAC	0.169	0.005	0.015	0.018		0.205
WNAP	0.230	0.000	0.024	0.104	0.027	

NOTE.—All nonzero values are significant after Bonferroni correction. SAA = South African aduncus type, ENA = Eastern North Atlantic, MED = Mediterranean, ENP = Eastern North Pacific, WNAC = Western North Atlantic Coastal, and WNAP = Western North Atlantic Pelagic.

Discussion

Of the killer whale populations sampled, all had significantly higher heterozygosity than expected under

Table 3
F_{ST} Values for Microsatellite DNA Loci (above Diagonal; from Hoelzel et al. 2007) and the DQB Locus (below the Diagonal) for the Killer Whale

	SR	AR	AT	CT	IC	BR
SR		0.067	0.137	0.141	0.145	0.077
AR	0.067		0.171	0.171	0.193	0.024
AT	0.206	0.031		0.037	0.145	0.154
CT	0.238	0.047	0.000		0.133	0.167
IC	0.132	0.043	0.087	0.144		0.195
BR	0.087	0.025	0.100	0.129	0.000	

NOTE.—All nonzero values are significant after Bonferroni correction. AR = Alaska residents, SR = Southern residents, CT = Californian transients, IC = Iceland, and BR = Berring Sea.

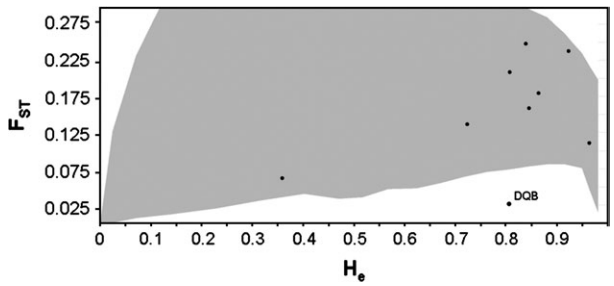

FIG. 3.—Plot of bottlenose dolphin loci comparing average H_e and F_{ST} for microsatellite DNA loci (after Natoli et al. 2005) and the DQB locus. Gray shading indicates the area on the graph within the confidence limits.

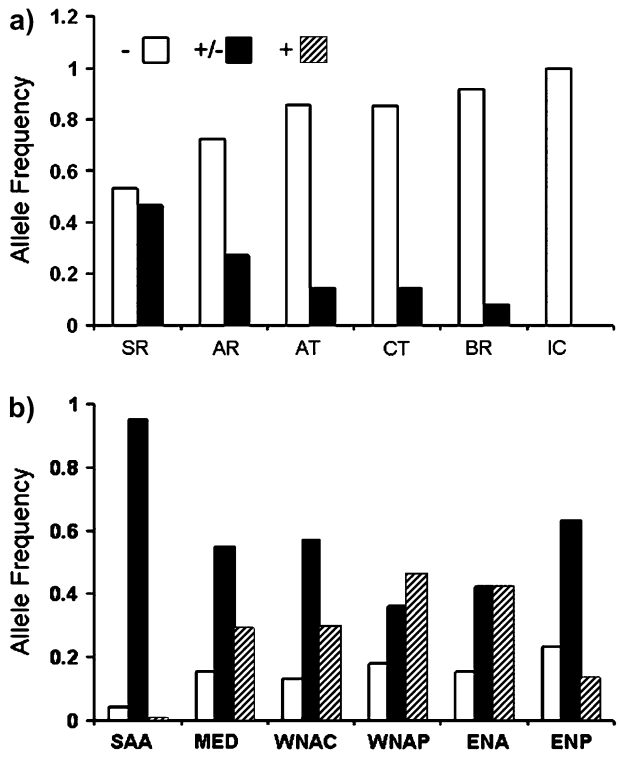

FIG. 4.—Categorization based on the total charge of Pocket 4 amino acid residues (β70, β71, and β74) in each population of (*a*) killer whale and (*b*) bottlenose dolphin.

Hardy–Weinberg equilibrium, consistent with the expectations of balancing selection. The Ewens–Watterson neutrality test was significant in half of the killer whale populations, similar to an HLA *DQB1* study where neutrality was rejected in nine populations out of 20 (Valdes et al. 1999). This test is based on the principle that rare allele advantage or selection for heterozygotes should lead to a more even distribution of allele frequencies than expected under neutrality (Ewens 1972). It has been suggested that populations not showing significant deviation from neutrality based on this test may be influenced by local directional selection (Sanchez-Mazas et al. 2000; Meyer and Thomson 2001); however, the Ewens–Watterson test is not very powerful when the number of alleles in the sample is relatively small, and therefore a failure to reject neutrality does not rule out balancing selection or drift (Fernandez-Vina et al. 1997; Meyer and Thomson 2001). The test is also sensitive to the assumption of mutation–drift equilibrium, which may not be the case for all tested populations.

Another expectation of balancing selection is that allele frequency differences among populations should be less pronounced at affected loci, reflected here in lower overall MHC F_{ST} values compared with the presumably neutral microsatellite loci. This effect was significant overall for the killer whale populations, though individual MHC F_{ST} values were quite high (and above the microsatellite F_{ST} distribution for two comparisons against the SR population). The pattern of differentiation showed no significant correlation for the two types of markers (based on both the Mantel test and the multiple regression analysis); however, outliers (see fig. 2) suggest that in addition to an apparent effect of balancing selection on all populations, there could be differential directional selection in local populations. If the MHC F_{ST} pattern was instead due to drift, it should be correlated to the pattern observed for the microsatellite loci, though the patterns could be due to drift and different for stochastic reasons. For the killer whale, there are two ecotypes represented by different populations included in this study, and it has been shown that different ecotypes diverge at neutral markers even in sympatry (Hoelzel et al. 2007). Based on microsatellite DNA, an isolation by distance pattern is seen only within the "resident" ecotype (Hoelzel et al. 2007). When comparisons using the MHC locus are restricted to within the resident ecotype, there is still no correlation between MHC and microsatellite F_{ST} values.

Our effort to investigate functional differences (without knowing specifically what this may correlate to) focused on pocket P4 of the DQB PBR. For the killer whale, only the SR population was significantly different. This population also showed the highest average MHC F_{ST} value in comparison with all other populations (0.146 vs. 0.043–0.112), and the only two pairwise comparisons that were higher than the microsatellite DNA F_{ST} 95% CI distribution. These convergent results reinforce the idea of differential directional selection in the SR population. The similarity among the other populations may reflect an overall pattern of balancing selection. It is interesting that comparisons between ecotypes do not represent uniformly higher MHC F_{ST} values or any evidence for functional differences based on the pocket P4 data, though this may simply reflect a lack of relevance for this locus and these comparisons.

The pattern for the *Tursiops* species was somewhat different. The best sampled population (*T. aduncus* in South Africa) showed a result similar to that seen for the killer whale populations, though the Ewens–Watterson test showed no significant deviation from neutrality for this population. Given the large sample size (and therefore relatively high power, though the allele number was not greater), this may suggest either drift or directional selection in this population. Some of the differences among the *T. truncatus* populations may be affected by sampling effects, but strong heterozygote deficiency was seen for two relatively well-sampled populations in the western North Atlantic. This could come about through strong selection or inbreeding. We suggest that selection is more likely in this case, because heterozygote deficiency was not seen at microsatellite loci for these populations (see Natoli et al. 2004, 2005).

For comparisons among populations of *T. truncatus*, the MHC F_{ST} values were significantly lower than those for microsatellite loci, suggesting balancing selection. This was also supported by the FDIST analysis, where the DQB locus showed significantly lower mean F_{ST} than predicted from its mean heterozygosity (fig. 3). However, as for the killer whale, the Mantel test and multiple regression data showed a lack of correlation between MHC and neutral loci, suggesting the possibility of differential directional selection. In this context, there were some interesting patterns revealed by the pocket P4 charge profiles. The nearshore and offshore populations in the western North Atlantic

are known to be affected by different pathogen species. In particular, *Phyllobothrium*, *Monorhygma*, and *Crassicauda* are found only in the offshore form, whereas *Braunina* is found in the coastal population (Wang et al. 1994). *Braunina* species also infect nearshore populations elsewhere in the world, including in the Mediterranean–Black Sea region (see Birkun 2002), and off the coast of Argentina (Sanchez et al. 2002). Nearshore and offshore samples in this study showed significantly different pocket P4 profiles. Furthermore, the two known nearshore populations (on either side of the North Atlantic) showed nearly identical pocket P4 profiles and a low MHC F_{ST} (0.015; second lowest of all pairwise comparisons involving these populations), but high F_{ST} based on microsatellite loci (0.221; fig. 3, table 4). The South African *T. aduncus* profile was significantly differentiated from all *T. truncatus* profiles, but showed a dominance of the +/− charge state also seen in the *T. truncatus* nearshore population profiles. *Tursiops aduncus* is dependent on nearshore habitat, though we do not know how similar the pathogen environment is for the two species. Although the possible pathogen-specific interactions are not known, the pattern of differences and similarities is consistent with an interpretation of directional selection and suggests a useful focus for future studies.

Studies of mate preference, especially in mice and humans, have shown that this may also affect MHC polymorphism, for example, suggesting that nonrandom mating in mice is correlated to MHC incompatibility (Egit and Brown 1989; Potts et al. 1991). In humans, Ober et al. (1997) showed that Hutterite mate choice is influenced by HLA haplotypes, with people avoiding spouses with the same haplotype as their own. MHC recognition in mice and humans is suggested to be determined through olfaction (Wedekind et al. 1995; Wedekind and Furi 1997). Although there may be a mechanism (e.g., taste) whereby cetaceans may be able to mate assortatively based on MHC genotype, there are no data to indicate this happens to date. The effect on diversity among populations would be similar to that of balancing selection.

For these highly mobile marine species, the expectation would be for panmixis across broad geographic ranges, but various studies have shown restricted gene flow over a range of hundreds or even tens of kilometers, especially for these two species (Natoli et al. 2005; Hoelzel et al. 2007). Differentiation by drift could therefore be expected at MHC loci, and cannot be excluded, but the stronger indications from this data set reflect both the long-term unifying effects of balancing selection, and local, differentiated populations that suggest directional selection.

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